METHOD FOR PRODUCING BEER

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method of
5   producing beer, particularly of filtering beer through a
filtration medium and cleaning the filtration medium with
enzymes such that it can be reused in beer filtration.

BACKGROUND OF THE INVENTION

10   In view of the extended marketing channels, germs
(e.g., bacteria) have to be removed from the beer in
order to make it storable. Nowadays, germ removal is
mainly carried out by pasteurization of the beer. To
this end, the beer is, for example, bottled or canned,
15   and heated to a temperature of between 62 and 69 °C to
kill the germs.

    This pasteurization does, however, involve
considerable energy consumption. It has the further
disadvantage that the energy introduced can trigger
20   chemical reactions which impair the product and are
difficult to control. These reactions can, for example,
adversely affect the flavor of the product ("pasteurized
taste"), and there is also the danger that undesired
substances will form. Pasteurization is, therefore, a
25   relatively expensive germ removing method involving high
energy expenditure and, consequently, having harmful
effects on the environment as well as reducing the
quality of the product.

    Another known germ removing method is cold-
30   filtration. Cold-filtered beer is available as so-called
"draft beer" in, for example, the United States, Japan
and Korea. This beer is prohibited in Europe because it
contains technical enzymes.

    These technical enzymes are present in the beer to
35   counteract a drawback inherent in the cold-filtration
method: early clogging of the filter. This clogging is
due to deposits of substances to be filtered out of the

beer on the upstream side of the filter, e.g., a membrane filter. The deposits are difficult or even impossible to remove from the filter and reduce the service life of the filter. This increases the cost of producing the beer as
5 membrane filters are expensive.

To prolong the service life of the filter, the manufacturers of membrane filters recommend cleaning the used membranes by treating them with proteases, glucanases, and xylanases, as well as with chemicals such
10 as surfactants, acids/bases, and oxidizing agents, to make them reusable. This cleaning can be carried out at, for example, two stages, with the above-mentioned enzymes at a first stage, followed optionally by additional cleaning with the above-mentioned chemicals in a second
15 stage.

The literature also discloses methods of cleaning membrane filters used in filtering beer, which cleaning methods involve a variety of techniques. For example, U.S. Patent 5,227,819 discloses a method for the cleaning
20 of a polyamide microporous membrane used in cold-filtering beer by passing a dilute alkaline solution through the microporous membrane. International Patent Application WO 96/23579 discloses a somewhat different method of cleaning a membrane filter used in beer
25 filtration. That method is characterized by treating the membrane filter with an enzyme-containing aqueous solution of β-glucanases, xylanases, and cellulases, cleaning the membrane filter with an acidic aqueous cleaning solution, and cleaning the membrane filter with
30 a peroxide-containing alkaline cleaning solution.

Given, for example, a filter area of approximately 320 m², a cleaning procedure will, by way of example, make provision for enzymatic cleaning after every 5,000 hectoliters filtered and an additional chemical cleaning
35 after every 20,000 hectoliters filtered. The typical service life of filters with the above-mentioned filter area of approximately 320 m² having undergone the

manufacturer-recommended cleaning is approximately
100,000 hectoliters.

The previously known cleaning procedures do,
however, have the disadvantage that they are unable to
5   remove the deposits on the filter to a satisfactory
extent, which causes the cleaning efficiency to diminish
strongly as the membrane filter increases in age.

Yet another disadvantage is the sudden, random
clogging of the filter membrane, unrelated to standard
10 norms like total nitrogen content, or percent of original
wort. A fully clogged membrane filter cannot be
satisfactorily cleaned under procedures following the
current state of technology, which greatly reduces the
service life of the filter. It is difficult to determine
15 when a filter will become so clogged that it cannot be
satisfactorily cleaned, and, therefore, a filter may be
cleaned prematurely or not in time, i.e., too early or
too late.

In view of the foregoing problems, there exists a
20 need for an improved method of producing beer,
particularly wherein the beer can be filtered through a
filtration medium that can be satisfactorily cleaned and
reused. The present invention provides such a method.
These and other advantages of the present invention, as
25 well as additional inventive features, will be apparent
from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a method for
30 producing beer comprising filtering beer through a porous
membrane until such time that the porous membrane is in
need of cleaning, contacting the porous membrane with an
enzyme selected from the group consisting of cellulases,
amylases, and combinations thereof, particularly a
35 cellulase having a crystalline:soluble cellulose activity
ratio at 60 minutes of at least about 0.1, to clean the
porous membrane, and then reusing the porous membrane to

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continue filtering beer. The present invention further provides a method for producing beer comprising filtering beer through a porous membrane that progressively clogs during filtration, monitoring the streaming or zeta

5   potential of the porous membrane as a measure of the extent of clogging of the porous membrane, halting filtration of the beer through the porous membrane before the porous membrane becomes fully clogged as determined by the streaming or zeta potential of the porous

10   membrane, cleaning the porous membrane, and then reusing the porous membrane to continue filtering beer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of beer filtration amount (g)

15   versus filtration time (sec) in connection with filtering beer through a previously unused, i.e., a new, porous membrane.

Figure 2 is a graph of beer filtration amount (g) versus filtration time (sec) in connection with filtering

20   beer through a clogged porous membrane.

Figure 3 is a graph of beer filtration amount (g) versus filtration time (sec) in connection with filtering beer through a previously clogged porous membrane cleaned in accordance with a prior art technique.

25   Figure 4 is a graph of beer filtration amount (g) versus filtration time (sec) in connection with filtering beer through a previously clogged porous membrane cleaned in accordance with the present invention.

Figure 5 is a schematic diagram depicting a device

30   for measuring the zeta potential of a filtration medium.

Figure 6 is a graph of filtration medium zeta potential (mV) versus electrolyte solution pH, wherein curve "a" is for a new porous membrane, curve "b" is for a porous membrane that has been partially clogged in

35   connection with filtering beer, and curve "c" is for a porous membrane that has been nearly fully clogged in connection with filtering beer.

Figure 7 is a schematic diagram depicting an
apparatus for filtering beer using a bypass system and
the measuring device of Fig. 5.

5   DESCRIPTION OF THE PREFERRED EMBODIMENTS
          The present invention provides a method for
producing beer, preferably cold-filtered beer. The
method comprises filtering beer through a porous
membrane, i.e., a membrane filter, until such time that
10   the porous membrane is in need of cleaning, contacting
the porous membrane with an enzyme to clean the porous
membrane, and then reusing the porous membrane to
continue filtering beer.
          It surprisingly has been discovered that porous
15   membranes can be cleaned better and more gently with a
cellulase and/or with an amylase than with proteases,
xylanases, and/or glucanases. Cleaning in accordance
with the present invention results in a considerable
increase in the service life of porous membranes used in
20   the filtering of beer and therefore greatly improves the
commercial benefit attendant the use of porous membranes
in the production of beer.
          The enzyme is selected from the group consisting of
cellulases, amylases, and combinations thereof. As
25   indicated above, proteases, xylanases, and/or glucanases
need not be used, and, preferably, are not used, with the
cellulase and/or amylase to clean the porous membrane.
The cellulase desirably has a crystalline:soluble
cellulose activity ratio (described more fully below) of
30   at least about 0.1, more desirably at least about 0.3,
preferably at least about 0.4, more preferably at least
about 0.5, and most preferably at least about 1,
particularly at least about 1.2. Suitable cellulases
include cellulases derived from *Aspergillus*, particularly
35   *Aspergillus niger*. Preferred cellulases include
cellulases derived from *Trichoderma*, preferably
Trichoderma reesei and *Trichoderma longibrachiatum*, and

Thermomonospora, preferably from *Thermomonospora fusca*.
Other sources of cellulases are recited in U.S. Patent
4,912,056. Suitable amylases include α-amylase, β-
amylase, and combinations thereof. More preferably, no

5   enzymes other than cellulases and amylases are utilized
in the present inventive method, i.e., the porous
membrane is not contacted with an enzyme other than a
cellulase or an amylase. Most preferably, the enzyme
utilized in the present inventive method is a cellulase,

10 and optimally no enzyme other than a cellulase is
utilized, i.e., the porous membrane is contacted with a
cellulase and is not contacted with any other enzyme.

        The porous membrane can be any membrane suitable for
the filtration of beer. In the context of the present

15 invention, the porous membrane typically will be a
microporous membrane, i.e., a porous membrane with a pore
rating of about 0.02-1 μm. The porous membrane
preferably will have a pore rating of about 0.1-1 μm,
most preferably about 0.45 μm. Such a porous membrane

20 can be used to remove bacteria and other undesirable
germs from the beer, preferably obviating the need to
pasteurize the beer. The porous membrane also can be
used to remove yeast and other undesirable substances
from the beer. Suitable porous membranes include those

25 prepared from inorganic materials such as ceramics and
metals, as well as, preferably, organic polymers such as
polyamides, polyethersulfones, polyolefins,
polyvinylidenefluoride, and the like. The porous
membrane preferably is a polyamide porous membrane,

30 especially a nylon-6,6 porous membrane.
        A preferred embodiment of the method according to
the present invention is characterized in that the porous
membrane is additionally brought into contact with an
aqueous base, with the porous membrane being

35 advantageously brought into contact with the aqueous base
at a first stage and with the enzyme at a second stage.
The use of an aqueous solution of NaOH and/or KOH as the

aqueous base has proven expedient. It is preferable for
the base to be present in a concentration of 0.1 to 1 N,
more preferably 0.25 to 1 N, and most preferably 0.5 to 1
N. The treatment with the aqueous base is best carried

5   out at a temperature of between 40 and 90 °C.

Further advantageous embodiments of the method
according to the present invention are characterized in
that the treatment with the cellulase is carried out at a
temperature of between 40 and 50 °C and a pH of between

10 4.5 and 5.5, the treatment with the α-amylase is carried
out at a temperature of between 60 and 75 °C and a pH of
between 4.6 and 5.8, and the treatment with the β-amylase
is carried out at a temperature of between 40 and 60 °C
and a pH of between 4.6 and 5.8.

15 The cleaning ~~It is expedient for the cleaning to be carried out~~ can be
until a point in time at which there is ~~no~~ more change in
the streaming potential or the zeta potential of the
porous membrane. It has been discovered that the
streaming potential occurring at the porous membrane

20 during operation or the zeta potential calculated from it
(see below) is a good indication of the extent to which
the substances clogging the porous membrane have been
~~removed.~~

The present invention also aims at increasing the

25 porous membrane's service life by ensuring that it is
cleaned at a desirable time. Thus, the present invention
provides for the production of beer comprising filtering
beer through a porous membrane, which will clog
progressively as filtration proceeds. Filtration is

30 halted at a given point when the porous membrane is only
partially clogged, i.e., has not yet reached the
condition of being totally clogged. The degree of
clogging can be determined by any suitable means,
desirably by monitoring the pressure drop across the

35 porous membrane such as is generally described in U.S.
Patent 5,449,465. Alternatively, the present invention
provides for an identification of the time for cleaning

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by determination of the streaming potential through the filter and/or zeta potential of the filter.

This aspect of the present invention is founded on the recognition that streaming potential -- or zeta

5   potential extrapolated from the former's recorded data -- will change in a pH range (within which beer brewing or filtering occurs) according to the degree of clogging and thus represents a reliable, and almost quantitative, indicator of the state of clogging. Determination of the

10 streaming potential and/or zeta potential of the porous membrane can hence give an accurate picture of a particular state of clogging.

Porous membranes are known to act in a two-fold way. First, a porous membrane acts as a sieve, when particles

15 larger then the filter's pores are mechanically filtered out of the medium. Secondarily, a porous membrane also is known to act by electrostatic attraction. Particles of a diameter much smaller than the pore size of the membrane are deposited thereon when the zeta potential of

20 the filter medium and that of the particles are of opposite polarity (see, e.g., Informational Brochure SD 872h G of Pall Filtrationstechnik GmbH, Germany).

Yet, not known prior to the present invention is the fact that zeta potential can be used to determine a

25 porous membrane's degree of clogging.

A porous membrane's zeta potential will be affected by its chemical properties. One of ordinary skill in the art ~~The expert~~ will have no difficulty -- being cognizant of the present invention -- to select only filters whose zeta potential will change

30 at a great enough rate relative to the degree of clogging. With the filter on-line, and by way of continuous monitoring through data acquisition, the filtration process can be halted at an appropriate time, e.g., once clogging sets in.

35 The cleaning of a filter not yet fully clogged is much easier, while assuring longer service life, than the cleaning of a totally clogged filter. Thus, a preferred

method of the present invention has filtration halted at a point when the filter's zeta potential has decreased to a maximum of 20% of the value it exhibited in its unused state, or when clogging does not exceed 80%.

5   Another refinement of the process will use a porous membrane of polyamide, with filtration halted when the zeta potential exceeds -5 mV as measured at a pH of 4.2.

The beer preferably will undergo pre-filtration before filtration proper, i.e., filtration through the

10   porous membrane. Diatomateous (or infusorial) earth, also known as diatomite, is almost exclusively used for pre-filtration. A combination of diatomateous earth and deep-bed filtration also is feasible.

The present invention can be used in any suitable

15   beer production system. Preferably, the present invention is used in connection with the cluster filter system as described in U.S. Patents 5,417,101 and 5,594,161.

The present invention also relates to a filtration

20   unit for filtering beer, with a feeder line for the filtration-bound beer, a porous membrane, and a run-off line for the filtered beer. It is characterized by a module in the form of a meter cell, functioning as bypass, and featuring a porous membrane and means, e.g.,

25   electrodes, for monitoring the streaming potential and/or zeta potential of the meter cell's membrane filter through which beer flows.

The present invention also deals with a filtration unit for filtering beer, with the unit featuring a feeder

30   line for filtration-bound beer, a porous membrane, and a run-off line for filtered beer. In divergence from the foregoing paragraph, the filtration unit is characterized by means, e.g., electrodes, being attached to the porous membrane for monitoring or reading the streaming

35   potential and/or zeta potential as the beer flows through the porous membrane. In this variation, the zeta potential is not measured via the meter cell assigned as

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bypass to the membrane filter, but rather on the membrane filter itself.

Any suitable bypass configuration can be utilized in connection with the embodiments of the present invention.

5   Preferably, the present invention incorporates the apparatus and method described in U.S. Patent 5,449,465.

The discovery that the filter's zeta potential correlates to the general state of clogging can be implemented in beer filtration as follows:

10   1.   Through constant observation of changes taking place in the streaming potential and/or zeta potential of the porous membrane during the filtration process, the membrane's degree of clogging can be pinpointed in order to prevent an unexpected or random occurrence, while

15   timely measures for an exchange of filters can be taken.

2.   Filtration can be halted before the porous membrane becomes totally clogged. This promotes easier cleaning of the filter. It has been shown that the clogging substances in a totally clogged filter can only

20   be removed with the greatest of difficulty by conventional methods of cleansing, or cannot be removed from the filter at all, resulting in abbreviated service life.

Once filtration is halted prior to total clogging,

25   the process of cleaning is much easier and more thorough, with the filter retaining an extended life. In the instance of a polyamide porous membrane, it has been discovered that the successful removal of all clogging substances from the porous membrane can be accomplished

30   when filtration is halted at a point where the zeta potential has not lost more than about 80% of its original value, i.e., is not clogged in excess of 80%.

3.   The cleaning method's success can be tested by determining the cleaned membrane's zeta potential. The

35   act of cleaning will return the zeta potential to approximately its original value.

By this procedure, the cleaning process can be evaluated and/or optimized for it's efficiency:

4. The aging of a porous membrane for reasons of repeated use can be tracked, providing a handy estimate as to its remaining service life expectancy.

5. By measuring zeta potential, filter material and shunting materials (e.g., diatomite, bentonite, perlite, polyvinyl pyrrolidone) can be tested for suitability in beer filtration by assessing the interaction between clogging substances of liquid systems and filter material and/or shunting means for filters.

6. The service life of a porous membrane can be estimated by way of measuring zeta potential, whereunder a specific membrane load (hl/m^2) is recorded up to the point when clogging sets in.

The artisan is aware that most suitable for the process are porous membranes with a zeta potential exhibiting pronounced change in relation to the degree of clogging. Verification of these parameters is easy enough by employing the aforementioned simple test method.

The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

This example illustrates the effectiveness of the present inventive method to produce beer. In particular, this example demonstrates that cellulases and amylases can be used to satisfactorily clean a porous membrane clogged in the course of beer filtration such that the porous membrane can be reused in continued beer filtration.

A porous membrane made of nylon-6,6 (NB type, commercially available from Pall Filtrationstechnik GmbH, Germany) was used as a filter. Such a filter is frequently used in the state of the art for the cold-filtration of beer.

The so-called membrane filter test according to Esser (Monatszeitschrift für Brauerei (Monthly Magazine for Breweries), 25th year, No. 6, pages 145-151, 1972) was used to determine the filtering performance of the filter. This test is reliable for checking measures for improving filterability.

To determine the filtering efficiency of a new, i.e., unused, porous membrane, a pressure filtration apparatus (SM 16526 type, 200 ml capacity; commercially available from Sartorius GmbH, Goettingen, Germany) was used for a polyamide nylon-6,6 porous membrane having a 47 mm diameter and a 0.2 µm pore size. *passed* ~~Beer cooled down to 0 °C was forced through the~~ porous membrane under isobaric conditions (1 bar), and the amount of filtrate was weighed every 10 seconds. The test was stopped after 200 g of filtrate were obtained. The result is shown as a graph in the diagram of Figure 1. Figure 1 shows that, under the conditions indicated above, the 200 g of filtrate were obtained with the ~~unused filter after approximately 210 seconds.~~

Under identical conditions, the filtering performance of a *partially* clogged, i.e., used, porous membrane was tested. The result is given in Figure 2 which shows that even in 720 seconds only approximately 60 g of filtrate were obtained.

The clogged porous membrane was cleaned in accordance with a prior art method, wherein the membrane was first cleaned enzymatically and then chemically, as described below.

For enzymatic cleaning, the clogged membrane was treated for 1 hour with a 1% aqueous solution of a mixture of β-glucanases and xylanases (P3-Ultrasil 65; commercially available from Henkel) with a pH of 5 (adjusted with a 0.05% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; commercially available from Henkel)) at a temperature of 50 °C. This treatment was subsequently carried out one more time.

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The membrane was then treated for 3 hours with a 0.5% aqueous solution of a mixture of surfactants, glucanases, and proteases (P3-Ultrasil 62; commercially available from Henkel) with a pH of 9-9.5 (set with a

5   0.15% aqueous solution of a mixture of surfactants and an alkaline component (P3-Ultrasil 91; commercially available from Henkel)) at a temperature of 50 °C and subsequently rinsed with warm water (50 °C).

For chemical cleaning, the membrane thereafter was

10   treated for 30 minutes with a 1% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; commercially available from Henkel) at 60 °C, and then rinsed with fresh water. The membrane was subsequently treated for 30 minutes with an aqueous

15   solution containing 1% of a mixture of surfactants and an alkaline component (P3-Ultrasil 91; commercially available from Henkel) and 1% of a mixture of surfactants and an oxygen donor (P3-Ultrasil 05; commercially available from Henkel) at a temperature of 60 °C and then

20   rinsed with fresh water. The membrane was then treated once more for 30 minutes with a 0.5% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; commercially available from Henkel) and subsequently rinsed with fresh water until the rinse

25   water reached the electrical conductivity of fresh water.

The filtering performance of this cleaned porous membrane was then tested again under the conditions indicated above. The result is shown in Figure 3. Figure 3 shows that the filtering performance has

30   improved somewhat as the 200 g of filtrate were obtained after approximately 600 seconds.

A similarly clogged membrane whose filtering efficiency is shown in Figure 2 was cleaned in accordance with the method according to the present invention. The

35   membrane was treated for 30 minutes with an aqueous solution of C_1- and C_x-cellulases, the solution having a pH value of 4.7, at a temperature of 45 °C. The membrane was then treated with the same solution, but at a pH value of 5.0 and a

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temperature of 50 °C, and, finally, at a pH value of 4.7
and a temperature of 60 °C for 60 minutes.

The membrane was subsequently rinsed with warm water
at 50 °C. The filtering performance of the membrane
5   cleaned in accordance with the present invention was
tested in accordance with the above procedure. The
result is shown in Figure 4.

Figure 4 shows that 200 g of filtrate were obtained
after approximately 220 seconds. This represents a
10 significant improvement over the prior art (Figure 3).
The method according to the present invention, therefore,
allows considerably better cleaning of a used membrane
filter than is possible with prior art cleaning methods.

Equally good results were obtained when, in
15 accordance with the present invention, an amylase was
used instead of a cellulase. The service life of a
porous membrane thus can be increased with the cleaning
method according to the present invention.

20   EXAMPLE 2

This example illustrates the use of the streaming or
zeta potential of a porous membrane to assist in the
cleaning of the porous membrane. In particular, the
streaming or zeta potential is demonstrated to be useful
25 in determining the extent of membrane cleaning as well as
when a membrane is most satisfactorily cleaned.

The zeta potential of membrane filters was
determined with the electrokinetic measuring system EKA
of Anton Paar GmbH, Austria. This measurement is based
30 on the streaming potential method. An electrolyte flows
through the filters, and the potential (streaming
potential) which is produced by shearing-off of
counterions is detected with electrodes, and the zeta
potential is calculated from this measured quantity (see
35 below).

Figure 5 shows diagrammatically the measuring cell
with which the streaming potential or the zeta potential

was determined.   Reference numeral 1 designates the measuring cell in which the porous membrane 2 is clamped without warping in filter holders 3 and 4 made of polytetrafluoroethylene.   The filter holders 3 and 4 are

5   the end pieces of two pistons 5 and 6, respectively, which are mounted for displacement in the cylindrical part 7 of the measuring cell 1.

The end pieces 3 and 4 of the pistons 5 and 6, respectively, have fine bores 10 and 11 for the fluid

10   which is to be filtered and press the perforated electrodes 8 and 9 against the porous membrane 2.   The electrodes 8 and 9 are connected to the two electric terminals 12 and 13 extending inside the pistons 5 and 6 so the streaming potential built up as fluid flows

15   through the membrane 2 can be measured.   Silver electrodes or silver chloride electrodes which exhibit a low polarization during passage of current are preferred for the electrodes.   The pistons 6 and 7 are mounted in the seals 14 and 15, respectively, such that, on the one

20   hand, they are displaceable, and, on the other hand, they do not allow any fluid to leak from the measuring cell.

The fluid to be filtered flows through the supply line 16 into the cylindrical part 7 of the measuring cell 1, through the fine bores 10 of the piston 6, through the

25   electrode 8, with an electric potential being built up, and through the porous membrane 2.   The filtered fluid flows through the electrode 9, with a potential likewise being built up, passes through the fine bores 11 of the piston and leaves the measuring cell through the

30   discharge line 17.

To determine the zeta potential from the measured streaming potential, measurement (not illustrated) of the differential pressure in the measuring cell between supply line 16 and discharge line 17, the conductivity

35   and also the pH value is necessary.   The zeta potential is calculated from these measured quantities as follows:

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$$\text{zeta potential} = \frac{U}{\Delta p} \cdot \frac{LF.\eta}{\varepsilon.\varepsilon_\circ}$$

5   where U is the streaming potential, Δp the pressure
difference, LF the conductivity, η the viscosity, and εε₀
the dielectric constant.

The change in the zeta potential of the membrane
filter as clogging progresses is shown in Figure 6. This
10   figure is a diagram in which the zeta potential in
millivolts is plotted as ordinate, and the pH value at
which the zeta potential was determined as abscissa. The
pH value of the electrolyte solution (0.001 N aqueous KCl
solution) was set with 0.1 N HCl or with 0.1 N NaOH. The
15   specified pressure difference was 350 mbar.

The diagram was obtained by first determining with
the measuring cell described above the zeta potentials of
a new, i.e., unused porous membrane made of polyamide (NB
type, commercially available from Pall Filtrationstechnik
20   GmbH, 6072 Dreieich 1, Germany) at various pH values.

The results relating to the unused porous membrane
are plotted as curve "a". It is evident that the unused
porous membrane has a zeta potential of approximately -18
mV with an alkaline pH, and that the zeta potential
25   increases with decreasing pH and finally reaches zero
value at a pH of approximately 3.

Curve "b" shows the dependence of the zeta potential
on the pH value of the porous membrane under identical
measuring conditions, as stated above, but after use
30   thereof for filtering beer and, therefore, with partial
clogging. As is apparent, the zeta potential is raised
somewhat by the partial clogging and only reaches a value
of approximately -15 mV at pH values of approximately 7.

Curve "c" was plotted for the same porous membrane
35   in the nearly fully clogged state. It is evident that
the zeta potential now changes only slightly with the pH
value, and even in the alkaline range does not fall below
approximately -2 mV.

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To test the cleaning according to the present
invention, the zeta potential of the membrane to be
cleaned is determined, and the cleaning was successful if
the zeta potential of the cleaned membrane shifted as far
5   as possible in the direction of the zeta potential of the
unused membrane.

It will be clear to one skilled in the art that
porous membranes whose zeta potential changes to a
sufficiently great extent as a function of the degree of
10   clogging are particularly well-suited for use in the
method according to the present invention. This
characteristic can be easily determined by one skilled in
the art by simple testing.

A porous membrane of polyamide is especially
15   suitable in the context of the process since the zeta
potential at the pH of the filtration-bound beer (ca. pH
= 4.2) will undergo severe change with progressive
clogging. As can be learned from Figure 6, the membrane
at this particular pH value at the beginning of
20   filtration shows a zeta potential of approximately -8 mV.
The totally clogged membrane has a zeta potential of
approximately -2 mV.

Figure 7 shows a variation of the discussed
filtration unit featuring a filtration chamber 18, with a
25   meter cell 22 assigned to it as a bypass, as depicted in
Figure 5. The filtration chamber 18 holds filter candles
19.

The filtration-bound beer is fed via line 20 into
the filtration chamber 18, flowing through the filter
30   candles (membrane filter) 19, and exits the filter
chamber 18 through run-off line 21 in the form of
filtered beer.

The meter cell is shown in Figure 7 without detail.
The actual flow through the meter cell 22 must be
35   controlled to the extent that an amount of beer is
filtered per cm^2 of the porous membrane's surface which is

equal to the amount of porous membrane surface per cm^2 in the filtration chamber 18.

subC4 The severe change in filter test membrane 2 (Figure 5) zeta potential inside meter cell 1 during filtration

5   allows an assessment of the state of the filter candles 19 in filtration chamber 18.

EXAMPLE 3

This example illustrates the effectiveness of

10 cellulase derived from *Aspergillus niger* in enzymatically degrading soluble and crystalline cellulose substrates.

Cellulase derived from *Aspergillus niger* was obtained from Fluka (item numbers 22178). The enzyme was evaluated with respect to two different celluloses: soluble

15 carboxymethylcellulose (CMC, available from Aldrich as item number 41927-3) and crystalline cellulose (Avicel, available from FMC as item number PH-105).

The test methodology involved the preparation of an incubation solution of (i) 18 ml CMC (1%) or Avicel (1%),

20 (ii) 5 ml sodium acetate buffer (50 mM, pH 4.8), and (iii) 5 ml of a solution of the enzyme in sodium acetate buffer (50 mM, pH 4.8) at 30 °C. A test solution then was prepared by mixing 1.4 ml of the incubation solution with 0.1 ml glucose solution (0.15%) and 1.5 ml 3,5-

25 dinitrosalicylic acid (DNS) reagent (available from Sigma as item number D-0550). The test solution was boiled for 15 minutes. The total μmol glucose equivalents/mg enzyme as a function of time (min) was determined spectroscopically (575 nm), using two parallel samples, in

30 accordance with the procedure described in Miller, *Anal. Chem.*, *31*, 426-28 (1959), using a straight line calibration with a glucose standard. Protein amounts were determined in accordance with the procedure described in Bradford, *Anal. Biochem.*, *72*, 248-64 (1976), using a

35 bovine serum albumin (BSA) standard.
The enzymatic degradation of cellulose results in the production of glucose, and, therefore, the measurement of

µmol glucose equivalents/mg enzyme is a measure of the
activity of the enzyme with respect to a particular type
of cellulose, e.g., soluble (CMC) or crystalline (Avicel)
cellulose.

5   The results of this evaluation with respect to the
cellulase derived from *Aspergillus niger* are set forth in
Table 1.   The test solution with the soluble (CMC)
cellulose substrate contained 0.8 mg enzyme/28 ml
incubation solution (ca. 17.6 µg protein). The test

10   solution with the crystalline (Avicel) cellulose substrate
contained 0.35 mg enzyme/28 ml incubation solution (ca.
7.7 µg protein).

Table 1:	Cellulase derived from *Aspergillus niger*		
Time (min)	Glucose Equivalents (µmol/mg enzyme)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--
10	27.0	0	0
15	28.5	1.7	0.06
30	30.5	--	--
45	34.0	1.7	0.05
60	34.8	4.0	0.11
75	37.5	3.5	0.09
90	37.8	3.7	0.10
105	38.3	--	--
120	39.5	10.3	0.26

15   Those enzymes that have a relatively greater activity
toward crystalline cellulose substrates as compared to
soluble cellulose substrates have been found to be
particularly effective in cleaning porous membranes used
in beer filtration. The ratio of the glucose equivalents

20   produced with respect to the crystalline cellulose
substrate and the glucose equivalents produced with

respect to the soluble cellulose substrate thus is an indicator of the effectiveness of the enzyme in the context of the present invention and is described as the crystalline:soluble cellulose activity ratio. Desirably,

5 the crystalline:soluble cellulose activity ratio has the previously described values at a range of times in the test protocol described in this example, e.g., at 30 minutes, 60 minutes, and/or 90 minutes, especially at 60 minutes.

10 As is apparent from the data set forth in Table 1, the cellulase from *Aspergillus niger* has a crystalline:soluble cellulose activity ratio at 60 minutes of 0.11, indicating that it is a moderately effective enzyme for purposes of cleaning porous membranes used in

15 connection with the filtration of beer.

EXAMPLE 4

This example illustrates the effectiveness of cellulase derived from *Trichoderma reesei* in enzymatically

20 degrading soluble and crystalline cellulose substrates.

Cellulase derived from *Trichoderma reesei* was obtained from Fluka (item numbers 22173). The enzyme was evaluated in the same manner as recited in Example 3.

The results of this evaluation with respect to the

25 cellulase derived from *Trichoderma reesei* are set forth in Table 2. The test solution with the soluble (CMC) cellulose substrate contained 0.37 mg enzyme/28 ml incubation solution (ca. 128 µg protein). The test solution with the crystalline (Avicel) cellulose substrate

30 contained 0.08 mg enzyme/28 ml incubation solution (ca. 25.6 µg protein).

Time (min)	Glucose Equivalents (μmol/mg enzyme)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--
5	62.6	0	--
10	84.7	21.5	0.25
15	96.3	30.0	0.31
30	99.5	40.0	0.40
45	152.0	57.5	0.38
60	139.0	75.0	0.54
75	178.4	85.0	0.48
90	184.2	95.0	0.52
105	172.6	100.0	0.58
120	193.7	115.0	0.59

Table 2: Cellulase derived from *Trichoderma reesei*

As is apparent from the data set forth in Table 2,
the cellulase from *Trichoderma reesei* has a
crystalline:soluble cellulose activity ratio at 60 minutes

5   of 0.54, indicating that it is a superior enzyme for
purposes of cleaning porous membranes used in connection
with the filtration of beer.

EXAMPLE 5

10   This example illustrates the effectiveness of β-
cellulase derived from *Bacillus subtilis* in enzymatically
degrading soluble and crystalline cellulose substrates.

β-cellulase derived from *Bacillus subtilis* was
obtained from Fluka (item numbers 49106). The enzyme was

15   evaluated in the same manner as recited in Example 3.

The results of this evaluation with respect to the β-
cellulase derived from *Bacillus subtilis* are set forth in
Table 3. The test solution with the soluble (CMC)
cellulose substrate contained 14.4 mg enzyme/28 ml

20   incubation solution (ca. 8.3 μg protein). The test

solution with the crystalline (Avicel) cellulose substrate
contained 15.6 mg enzyme/28 ml incubation solution (ca.
8.8 µg protein).

Table 3: β-Cellulase derived from *Bacillus subtilis*			
Time (min)	Glucose Equivalents (µmol/mg enzyme)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--
5	1.1	0.1	0.09
10	1.0	0.1	0.10
15	0.9	0.1	0.11
30	0.9	0.1	0.11
45	1.0	0.1	0.10
60	1.0	0.1	0.10
75	1.0	0.2	0.20
90	1.1	0.2	0.18
105	1.1	0.1	0.09
120	1.3	0.1	0.08

5   As is apparent from the data set forth in Table 3,
the β-cellulase from *Bacillus subtilis* has a
crystalline:soluble cellulose activity ratio at 60 minutes
of 0.10, indicating that it is a moderately effective
10   enzyme for purposes of cleaning porous membranes used in
connection with the filtration of beer.

EXAMPLE 6

     This example illustrates the effectiveness of
15   exocellulase derived from *Thermomonospora fusca* in
enzymatically degrading soluble and crystalline cellulose
substrates.

     Exocellulase E3 derived from *Thermomonospora fusca*
was obtained from Cornell University. The enzyme was
20   evaluated in the same manner as recited in Example 3

·23

except that the incubation solution comprised (i) 18 ml CMC (1%) or Avicel (1%), (ii) 9 ml sodium acetate buffer (50 mM, pH 5.6), and (iii) 1 ml of a solution of the enzyme in sodium acetate buffer (50 mM, pH 5.6), shaken at
5   50 °C (ca. 960 μm protein). The test solution was evaluated using a color test rather than the DNS test recited in Example 3.

The results of this evaluation with respect to the exocellulase derived from *Thermomonospora fusca* are set
10   forth in Table 4.

Table 4: Exocellulase derived from *Thermomonospora fusca*			
Time (min)	Glucose Equivalents (μmol/mg enzyme)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--
5	0.1	0	--
10	0.1	0.3	3.00
15	0.2	0.3	1.50
30	0.2	0.3	1.50
45	0.3	0.4	1.33
60	0.3	0.4	1.33
75	0.3	0.5	1.67
90	0.3	0.3	1.00

As is apparent from the data set forth in Table 3, the β-cellulase from *Bacillus subtilis* has a
15   crystalline:soluble cellulose activity ratio at 60 minutes of 1.33, indicating that it is a superior enzyme for purposes of cleaning porous membranes used in connection with the filtration of beer.

EXAMPLE 7

This example illustrates the effectiveness of α-amylase derived from *Bacillus subtilis* in enzymatically degrading soluble and crystalline cellulose substrates.

5   α-amylase derived from *Bacillus subtilis* was obtained from Fluka (item numbers 10069). The enzyme was evaluated in the same manner as recited in Example 3 except that the incubation solution comprised (i) 18 ml CMC (1%) or Avicel (1%), (ii) 5 ml sodium acetate buffer (50 mM, pH 6.9), and

10   (iii) 5 ml of a solution of the enzyme in sodium acetate buffer (50 mM, pH 6.9), shaken at 30 °C (ca. 8.5 μm protein). The test solution was evaluated using a color test rather than the DNS test recited in Example 3.

The results of this evaluation with respect to the α-

15   amylase derived from *Bacillus subtilis* are set forth in Table 5.

Table 5:	α-Amylase derived from *Bacillus subtilis*		
Time (min)	Glucose Equivalents (μmol/mg enzyme)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--
5	0.1	0	0
10	0.1	0	0
15	0.1	0	0
30	0.1	0	0
45	0.1	0	0
60	0.1	0	0
75	0.1	0	0
90	--	0	--

As is apparent from the data set forth in Table 5,

20   the α-amylase from *Bacillus subtilis* has a crystalline:soluble cellulose activity ratio at 60 minutes of about 0 (<0.1 μmol detection limit), indicating that it

is not as effective an enzyme for purposes of cleaning
porous membranes used in connection with the filtration of
beer as the previously described cellulases.

EXAMPLE 8

5   This example illustrates the effectiveness of various
cellulases in enzymatically degrading soluble and
crystalline cellulose substrates.

Cellulase preparations were obtained from the Erbsloh
10   Company: (a) C_x-cellulase (powder, item number VP 0945/2),
(b) C_1-cellulase from *Trichoderma reesei* (powder, item
number VP 0965/2), (c) C_1-cellulase (liquid, item number
Cleanzym SB1), (d) C_1-cellulase (liquid, item number VP
0976/4), (e) cellulase (liquid, item number VP 0971/1),
15   and (f) cellulase (liquid, item number VP 0971/4). The
enzymes were evaluated in a manner similar to that recited
in Example 3 except that the incubation solutions
comprised (i) 23 ml CMC (1%) or Avicel (1%) in a sodium
acetate buffer (50 mM, pH 4.8), and (ii) 5 ml of a
20   solution of the enzyme in sodium acetate buffer (50 mM, pH
4.8). 0.5% stock solutions were prepared from the
powdered enzyme preparations (5 mg/ml) and liquid enzyme
preparations (5 µl/ml). The solutions were shaken at 30
°C. The test solution was evaluated after making a 1:5
25   dilution using a color test rather than the DNS test
recited in Example 3.

The results of this evaluation with respect to the
various cellulases are set forth in Table 6. The glucose
equivalents data is in terms of average µmol glucose
30   equivalents/min (for the total time interval) and are not
normalized per mg enzyme (as was the situation with the
data recited in Tables 1-5). The crystalline:soluble
cellulose activity ratio, of course, is not altered by the
units for the glucose equivalents inasmuch as the units
35   divide out in calculating the ratio (i.e., the ratio is
unit-less).

Table 6: Cellulase Preparations

Glucose Equivalents (µmol/min)

Time (min)	Preparation (a)			Preparation (b)			Preparation (c)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline:Soluble Cellulose Activity Ratio	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline:Soluble Cellulose Activity Ratio	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline:Soluble Cellulose Activity Ratio
0	0	0	--	0	0	--	0	0	--
5	0.12	0	0	0.14	0.11	0.79	0.09	0.05	0.56
10	0.06	0.01	0.17	0.11	0.08	0.73	0.09	0.06	0.67
15	0.06	0.01	0.17	0.08	0.09	1.13	0.08	0.04	0.50
30	0.02	0.02	1.00	0.07	0.05	0.71	0.07	0.03	0.43
45	0.04	0.02	0.50	0.06	0.04	0.67	0.03	0.02	0.67
60	0.03	0.02	0.67	0.04	0.03	0.75	0.05	0.02	0.40
75	0.03	0.02	0.67	0.04	0.03	0.75	0.04		--
90	0.02	0.01	0.50	0.03	0.03	1.00	0.03	0.2	0.67

Glucose Equivalents (µmol/min)

Time (min)	Preparation (d)			Preparation (e)			Preparation (f)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline:Soluble Cellulose Activity Ratio	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline:Soluble Cellulose Activity Ratio	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline:Soluble Cellulose Activity Ratio
0	0	0	--	0	0	--	0	0	--
5	0.01	0.23	23.0	0.15	0.13	0.87	0.07	0.04	0.57
10	0.19	0.14	0.74	0.10	0.09	0.90	0.06	0.03	0.50
15	0.15	0.12	0.80	0.06	0.05	0.83	0.04	0.02	0.50
30	0.10	0.09	0.90	0.05	0.03	0.60	0.03	0.01	0.33
45	0.07	0.07	1.00	0.04	0.03	0.75	0.02	0.01	0.50
60	0.06	0.06	1.00	0.03	0.02	0.67	0.02	0.01	0.50
75	0.05	0.06	1.20	0.03	0.02	0.67	0.02	0.01	0.50
90	0.04	0.06	1.50	0.03	0.02	0.67	0.01	0.01	1.00

As is apparent from the data set forth in Table 6, the various cellulases have crystalline:soluble cellulose activity ratios at 60 minutes ranging from 0.4-1.0,

5   indicating that they are superior enzymes for the purpose of cleaning porous membranes used in connection with the filtration of beer.

                              EXAMPLE 9

10   This example further illustrates the effectiveness of the present inventive method to produce beer. In particular, this example demonstrates that cellulases alone (i.e., without the use of other enzymes) are superior in the cleaning of porous membranes clogged in

15   the course of beer filtration for the purpose of returning the porous membrane to use in continued beer filtration.

Beer of different characteristics was filtered through nylon-6,6 porous membranes (ca. 300 m^2) with a

20   pore rating of 0.45 μm in a cluster filter arrangement (PALL-CFS, available from Pall Filtrationstechnik GmbH, Germany). At certain beer filtration intervals, the porous membranes were subjected to a cleaning process in accordance with the present invention.

25   The cleaning process involved circulation of a 0.5% NaOH solution for 15 minutes, followed by a 60 minute soak. The porous membranes then were backflushed with water. An internal loop was established through the cluster filter arrangement with water at 38 °C. Lactic

30   acid was added to the water to adjust the pH to 4.2 ± 0.3, and then 6 l of an enzyme preparation containing a cellulase derived from *Trichoderma longibrachiatum* obtained from the Erbsloh Company (item number VP 0945/1) was added to the water via a dosing pump. The enzyme

35   preparation in the water (at a concentration of about 20-40 g enzyme/100 kg filter housing fluid volume) was circulated for about 15 minutes, followed by a 30 minute

soak, another 15 minute circulation, and finally a 6 hour soak. The porous membranes then were backflushed with water.

5   The porous membranes were cleaned after about 90,000 hl total beer was filtered through the porous membranes, and then the porous membranes were returned to service, i.e., to continue filtering beer. The porous membranes similarly were cleaned and returned to service after about 100,000 hl, about 140,000 hl, and about 165,000 hl

10   total beer was filtered through the porous membranes. The porous membranes mechanically failed after about 190,000 hl total beer was filtered through the porous membranes.

        The foregoing data demonstrates that beer can be

15   satisfactorily produced using the present invention. Specifically, the results of this example demonstrate that a porous membrane can be effectively cleaned and returned to service in accordance with the present invention, thereby prolonging the useful life of the

20   porous medium in a beer production process.

        All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

25   While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as

30   specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.